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Abstract

Transmission of *Escherichia coli* O157:H7 among reservoir animals is generally thought to occur either by direct contact between a naïve animal and an infected animal or by consumption of food or water containing the organism. Although ruminants are considered the major reservoir, there are two reports of human infections caused by *E. coli* O157:H7 linked to the consumption of pork products or to the contamination of fresh produce by swine manure. The objective of this study was to determine whether *E. coli* O157:H7 could be transmitted to naïve animals, both sheep and swine, that did not have any direct contact with an infected donor animal. We recovered *E. coli* O157:H7 from 10/10 pigs with nose-to-nose contact with the infected donor or animals adjacent to the donor and from 5/6 naïve pigs that were penned in the same room as the donor pig but 10 to 20 ft away. In contrast, when the experiment was repeated with sheep, *E. coli* O157:H7 was recovered from 4/6 animals that had nose-to-nose contact with the infected donor or adjacent animals and from 0/6 naïve animals penned 10 to 20 ft away from the donor. These results suggest that *E. coli* O157:H7 is readily transmitted among swine and that transmission can occur by the creation of contaminated aerosols.

Disciplines

Veterinary Medicine | Veterinary Microbiology and Immunobiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

Comments

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Indirect Transmission of *Escherichia coli* O157:H7 Occurs Readily among Swine but Not among Sheep▽

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Transmission of *Escherichia coli* O157:H7 among reservoir animals is generally thought to occur either by direct contact between a naïve animal and an infected animal or by consumption of food or water containing the organism. Although ruminants are considered the major reservoir, there are two reports of human infections caused by *E. coli* O157:H7 linked to the consumption of pork products or to the contamination of fresh produce by swine manure. The objective of this study was to determine whether *E. coli* O157:H7 could be transmitted to naïve animals, both sheep and swine, that did not have any direct contact with an infected donor animal. We recovered *E. coli* O157:H7 from 10/10 pigs with nose-to-nose contact with the infected donor or animals adjacent to the donor and from 5/6 naïve pigs that were penned in the same room as the donor pig but 10 to 20 ft away. In contrast, when the experiment was repeated with sheep, *E. coli* O157:H7 was recovered from 4/6 animals that had nose-to-nose contact with the infected donor or adjacent animals and from 0/6 naïve animals penned 10 to 20 ft away from the donor. These results suggest that *E. coli* O157:H7 is readily transmitted among swine and that transmission can occur by the creation of contaminated aerosols.

Escherichia coli O157:H7 infections are an important cause of food-borne illness in much of the world. Human disease usually results from the contamination of food or water by ruminant manure, and cattle are considered to be the primary reservoir of Shiga toxin-producing *E. coli*, including serotype O157:H7. Over the last several years *E. coli* O157:H7 has been recovered from small numbers of healthy pigs in Japan (17), Canada (11), Sweden (9), and the United States (10, 14). Recently, a small cluster of human infections caused by *E. coli* O157:H7 were traced back to dry fermented pork salami as the source (6). In addition, a large outbreak of human cases in the United States was linked to spinach potentially contaminated by both feral swine and cattle manure (12). *E. coli* O157:H7 can be carried by experimentally infected swine for at least 2 months (3, 4), and we have shown that transmission between naïve animals penned with an infected donor occurs freely (3, 4, 8). The objective of the current study was to determine whether or not *E. coli* O157:H7 could be transmitted to naïve animals that did not have any direct contact with an infected donor animal.

(A preliminary report of this work was presented at the Annual Meeting of the Food Safety Consortium, Fayetteville, AR, and at the Annual Meeting of the American Society for Microbiology, Toronto, Canada, 2007.)

MATERIALS AND METHODS

Animals and pen arrangement. All animal experiments were done with the approval of the Institutional Animal Care and Use Committee of Iowa State University. Young adult pigs (100 to 150 lb) and sheep (80 to 120 lb) were purchased from various commercial sources and were acclimated to antibiotic-free feed (for the pigs, Lean Grow 100 [Land O' Lakes] was used; for the sheep, a commercial concentrate [1 lb/day] plus alfalfa/grass hay fed ad libitum was used) for 2 weeks. Animals were housed in a biohazard level 2 facility and penned as shown in Fig. 1A. In order to minimize the total number of animals used and to maximize the number of animals that did not have any physical contact with the donor, the pen arrangement shown in Fig. 1B was used for the final replication for each species (the third replication for pigs and the second replication for sheep). Pens were cleaned daily by first removing as much manure as possible using a shovel. Pens were then washed using a high-pressure hose, starting with pen 4 and working toward pen 1 while being careful not to let the water move back toward pen 4.

Inoculation and recovery of *E. coli* O157:H7. Donor animals were inoculated with 5×10^8 CFU of *E. coli* O157:H7 strain 86-24 or strain 3081 by mixing the challenge strain in a small amount of feed in a separate room and were monitored to ensure that the entire inoculum was consumed. The inoculum strain used depended on the antibiotic resistance of the background commensal flora. Previously we have shown that there are no significant differences between these strains in colonization of pigs and sheep (7, 8). Three days after inoculation the donor animal was moved in with the naïve animals and placed in pen 1 (Fig. 1). Individual fecal samples were collected from both donors and naïve animals on days 2, 3, 4, 14, 15, and 16 postexposure immediately after the pens were cleaned. Fecal samples were cultured as previously described (8). Briefly, 5-g samples were added to 20 ml of phosphate-buffered saline and mixed in a Stomacher blender, and then serial 10-fold dilutions were made using phosphate-buffered saline. Samples were directly inoculated in triplicate onto selective media (dulcitol MacConkey's agar containing 20 μg/ml nalidixic acid and 100 μg/ml streptomycin or sorbitol MacConkey's agar containing 30 μg/ml kanamycin and 100 μg/ml ampicillin). Enrichment cultures (10 g of feces in 100 ml Trypticase soy broth plus 0.02% bile salts) were incubated overnight at 37°C, concentrated using immunomagnetic beads (Dynabeads; Dynal, Oslo, Norway), and plated onto the selective medium described above (8). The sensitivity of the direct plating method was 50 CFU/g.

Necropsy. All animals were subjected to necropsy procedures 2 weeks postexposure, and the following types of tissue were collected from pigs: buccal cell, tonsil, stomach, jejunum, ileum, cecum, spiral colon, distal colon, and rectoanal junction. Samples collected from the sheep during necropsy included buccal cell, ileum, spiral colon, distal colon, cecum, and rectoanal junction tissues. All tissues (~10 g each) were cultured by use of direct plating and enrichment broth as described above.

Air samples. Air samples were collected three times per week using an SKC BioSampler (Impinger) before and after the pens were cleaned during the final replication of each experiment (see pen arrangement in Fig. 1B). Air samples were collected for 15 min, with a sample flow rate of 12.5 liters/min (16).

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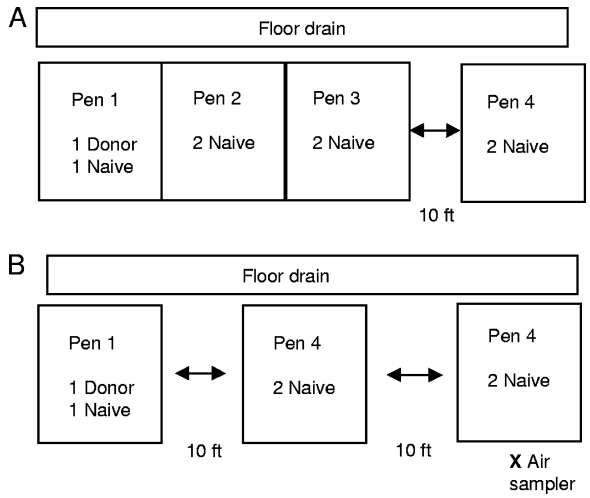

FIG. 1. Arrangement of animal pens during the first replication with sheep and first two replications with swine (A) and during the final replication (B).

RESULTS

E. coli O157:H7 was readily transmitted from the inoculated donor pigs to the majority of the naïve pigs in the same pen (pen 1) and in the adjacent pen (pen 2) during the first 2 to 4 days of exposure (Table 1). One pig each in pens 3 and 4 was also culture positive for *E. coli* O157:H7 during this time period. By 2 weeks postexposure, all of the pigs in pens 1 to 3 were shedding *E. coli* O157:H7 in their feces, as were five of the six pigs in pen 4. Shedding by donor pigs ranged from 8×10^2 to 8×10^4 CFU/g at the time that they were exposed to the naïve pigs (Fig. 2A). Shedding by the naïve pigs ranged from undetectable levels to 5×10^3 CFU/g during days 2 to 4 postexposure and from undetectable levels to 9×10^2 CFU/g at 2 weeks postexposure. At necropsy (2 weeks postexposure), *E. coli* O157:H7 was recovered from the tissues of approximately 35% of the pigs (Table 2). These included positive tissues (stomach, ileum, cecum, and rectoanal junction) from the lone pig in pen 4 that did not produce positive fecal cultures at this time. Air samples collected before cleaning (1/1) and after cleaning (2/3) during the first 2 to 4 days postexposure were positive (<50 CFU) for *E. coli* O157:H7 by enrichment only. Air samples collected over the next 2 weeks (five samples before cleaning and five after cleaning) were negative for *E. coli* O157:H7.

In contrast to what was seen in pig results, the transmission of *E. coli* O157:H7 among sheep was confined to the animals that had nose-to-nose contact with the inoculated donor animal and to one of two animals that were in pen 3 (Table 1). *E. coli* O157:H7 was recovered by enrichment cultures only from the naïve animals (Fig. 2B). None of the six sheep housed in pen 4 shed *E. coli* O157:H7 over the 2-week experimental period, and the organism was not recovered from any of the sheep tissues collected at necropsy. Donor sheep were shedding slightly higher numbers of *E. coli* O157:H7 (1×10^5 and 5×10^5 CFU/g) when they were exposed to the naïve animals. Air samples (nine taken before and nine after cleaning) from the second replication of the experiment were all negative for *E. coli* O157:H7.

DISCUSSION

The transmission of *E. coli* O157:H7 among both ruminants (5, 7, 15, 20) and swine (8) is well documented in situations where there is shared water and feed and/or nose-to-nose contact between infected individuals and naïve animals. Besser et al. (2) reported the transmission of *E. coli* O157:H7 between calves that were penned with a solid divider between them which prevented nose-to-nose contact. However, the dividers in the pens did not completely prevent small amounts of feces and urine from passing under them. In our experiments the location of pen 4, which was at least 10 feet away from the pens of all of the other animals, precluded the direct passage of contaminated feces from the infected donor to the naïve animals (Fig. 1). In addition, the pens were cleaned starting with pen 4 and ending with pen 1 and washing was directed with the airflow. Extreme care was taken not to wash contaminated waste toward the animals penned further away from the donor or against the airflow of the room. Despite these precautions, *E. coli* O157:H7 was recovered either from fecal cultures or from tissues collected at necropsy from all of the pigs in pen 4. This suggests that aerosol transmission of the organism occurred readily in this setting. Recovery of the inoculum strain from three air samples is further evidence that *E. coli* O157:H7 can be transmitted to swine via aerosols. Since the animal pens were washed using a high-pressure hose (after shoveling), this was the likely source of the contaminated aerosols even though the vast majority of the fecal material had been removed prior to washing. The length of time these aerosols could remain suspended would depend on the size of the particles generated and the relative humidity of the air within the room (21). The recovery of *E. coli* O157:H7 from an air sample 24 h after cleaning suggests that some infectious particles remained in suspension for at least that long and emphasizes the low infectious dose of *E. coli* O157:H7 for pigs. This work also confirms the report by Varma et al. demonstrating that inadvertent aerosolization of *E. coli* O157:H7 can result in transmission of the organism to humans or animals (22).

We cannot rule out the possibility that aerosol production by the infected swine themselves did not occur. Typical behavior in pigs during feeding and rooting can result in the deposition of contaminated food and feces in the nasal cavity (1, 18). It seems likely that this behavior would also produce infectious

TABLE 1. Transmission of *E. coli* O157:H7 between pens

Animal category and sample collection time	No. of animals with positive results/no. of animals exposed to infection				
	Donor	Pen 1	Pen 2	Pen 3	Pen 4
Pig					
Initial[a]	3/3	2/3	3/4	1/3	1/6
Wk 2	3/3	3/3	4/4	3/3	5/6
Sheep					
Initial	2/2	2/2	1/2	1/2	0/6
Wk 2	1/2	1/2	0/2	0/2	0/6

[a] Initial, days 2 to 4 postexposure.

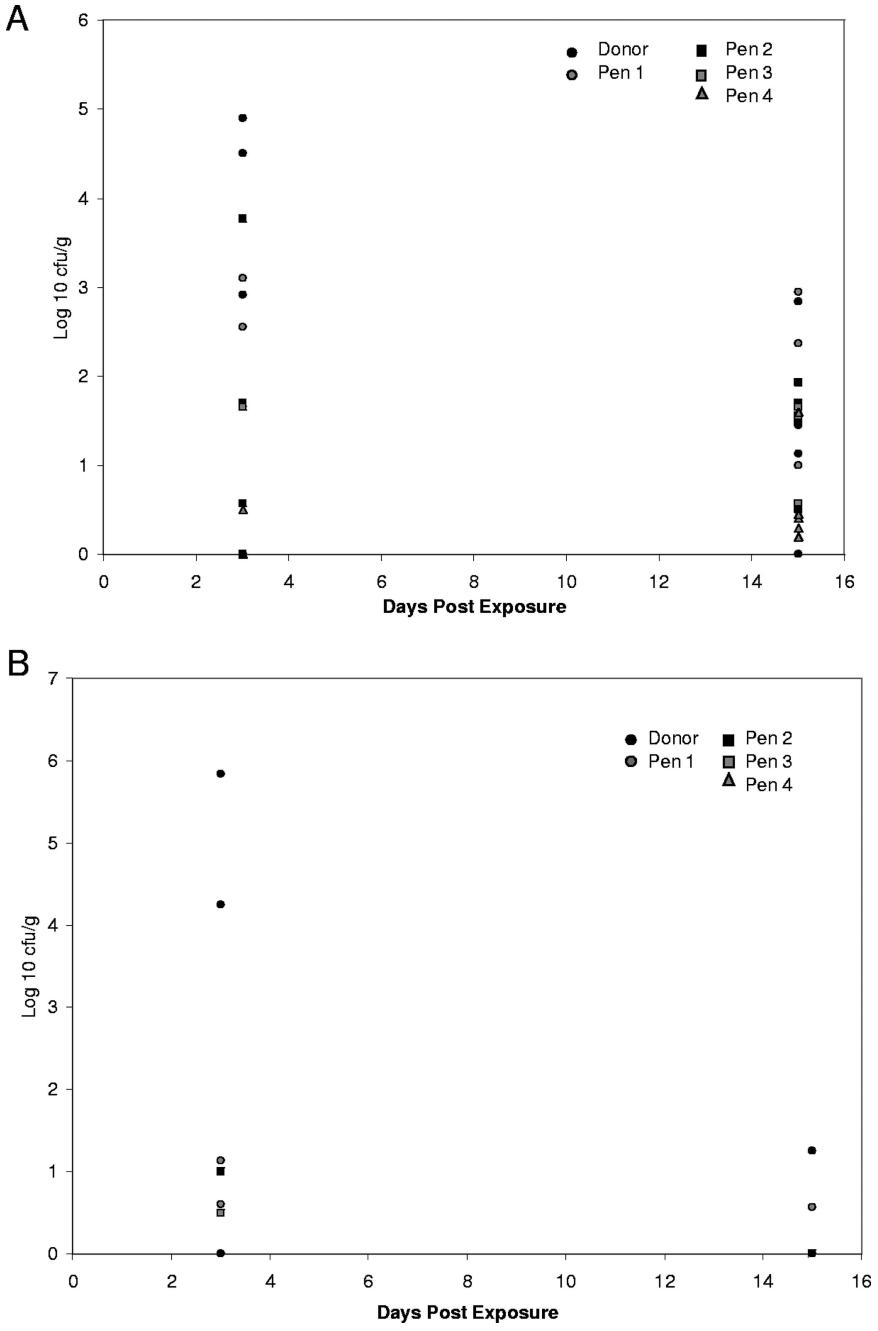

FIG. 2. Fecal shedding of *E. coli* O157:H7 by swine (A) and sheep (B) exposed to a donor animal shedding 10^4 to 10^5 CFU/g.

TABLE 2. *E. coli* O157:H7 recovered from swine tissues at necropsy

Tissue	No. of positive isolates/ total no. of isolates	Mean $\log_{10}$ CFU/g (range)
Buccal cells	3/19	1.7 (1.7)
Tonsil	5/19	1.7 (1.7)
Stomach	5/19	1.7 (1.7)
Jejunum	7/19	2.3 (1.7–5.4)
Ileum	7/19	1.9 (1.7–3.1)
Cecum	8/19	2.2 (1.7–3.9)
Spiral colon	7/19	1.8 (1.7–2.4)
Distal colon	7/19	1.8 (1.7–2.2)
Rectoanal junction	7/19	2.3 (1.7–4.4)

aerosols regardless of whether an enteric pathogen was already present in the oral cavity. We have shown previously that the tonsils of some pigs are colonized by significant levels of *E. coli* O157:H7 ($>10^3$ CFU/cm) and that the organism may also be present at low levels in the buccal cavity (Table 2) (8, 13). However, in these experiments only low levels (<50 CFU/cm) of *E. coli* O157:H7 were recovered from the tonsils of five pigs at necropsy (Table 2). It has been shown that *Salmonella enterica* can be transmitted among weaned piglets housed in isolation cabinets via contaminated aerosols (18) or between rooms that have a connected air space (19).

The lack of indirect transmission among the sheep in this experiment suggests that *E. coli* O157:H7 may not have been aerosolized as readily as it was in the experiments using pigs. Pens were set up and cleaned in the same manner in both experiments, and the donor animals were shedding similar amounts of *E. coli* O157:H7 during the first week (for pigs, mean 4.1 $\log_{10}$; for sheep, mean 5.0 $\log_{10}$ CFU/g). The apparent lack of infectious aerosols could be due to the inherent differences in the consistency of the feces of each species. Even though the pens of both species were cleaned as much as possible prior to washing, it is likely that more residual material remained in the swine pens. There are also marked differences in animal behavior between sheep and pigs that could account for the contrasting results we found. It would be of great interest to repeat this experiment using cattle, which are considered the major host reservoir for *E. coli* O157:H7 and the source, either directly or indirectly, of most human infections.

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